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☐ 1. Document ID: US 6232445 B1

L1: Entry 1 of 3 File: USPT May 15, 2001

DOCUMENT-IDENTIFIER: US 6232445 B1
TITLE: Soluble MHC complexes and methods of use thereof

DEPR:
Methods for the immunoaffinity purification of MHC class II molecules have been described previously (Gorga, J. C., V. Horejsi, D. R. Johnson, R. Raghupathy, and J. L. Strominger. (1987) J. Biol. Chem. 262:16087). These methods can be generally employed to purify soluble sc-MHC class I or II proteins of the invention. For example, for sc-MHC class II fusion proteins carrying HLA-DR or HLA-DQ domains, the monoclonal antibodies L243 and G2a.5 (immunospecific for DR and DQ, respectively, and available from ATCC) can be used to immunopurify sc-MHC class II molecules which include these domains. In one example, these methods were employed to purify the sc-DR2.DELTA..beta.2/MBP molecules produced in insect cells (see Example 5). The results of such a purification are shown in FIG. 5B.

CLPR:
1. A sc-MHC class II fusion protein comprising a recombinantly fused polypeptide comprising: i) a presenting peptide and ii) a class II .beta.2 chain comprising at least one amino acid substitution or deletion; wherein the .beta.2 chain increases expression of the fusion protein relative to sc-MHC class II fusion protein comprising the class II .beta.2 chain without the amino acid substitution or deletion.

CLPR:
2. The sc-MHC class II fusion protein of claim 1 further comprising an immunoglobin light chain constant region or fragment thereof.

CLPR:
6. A sc-MHC class II fusion protein comprising a recombinantly fused polypeptide comprising i) a presenting peptide and ii) a immunoglobin light chain constant region or fragment thereof; wherein the immunoglobin light chain constant region or the fragment increases expression of the fusion protein relative to the sc-MHC class II fusion protein without the immunoglobin light chain constant region or fragment.

CLPR:
20. A sc-MHC class II fusion protein comprising covalently linked in sequence:

CLPL:
wherein the increase in expression is relative to sc-MHC class II fusion protein comprising: i) the presenting peptide, ii) the MHC class II .beta.1 chain or presenting-peptide binding portion thereof, iii) the peptide linker

sequence, iv) the MHC class II .alpha.1.alpha.2 chain or a presenting-peptide binding portion thereof, and v) the class II .beta.2 chain without the amino acid substitution or deletion.

CLPL:
wherein the increase in expression is relative to sc-MHC class II fusion protein comprising: i) the presenting peptide, ii) the MHC class II .beta.1 chain or presenting-peptide binding portion thereof, iii) the peptide linker sequence, and iv) the MHC class II .alpha.1.alpha.2 chain or a presenting-peptide binding portion thereof, with the proviso that the sc-MHC class II fusion protein not comprise the immunoglobin light chain constant region or fragment.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 2. Document ID: US 6211342 B1

L1: Entry 2 of 3 File: USPT Apr 3, 2001

DOCUMENT-IDENTIFIER: US 6211342 B1
TITLE: Multivalent MHC complex peptide fusion protein complex for stimulating specific T cell function

DEPR:
The fusion protein can be prepared by constructing a gene which encodes for the production of the fusion protein. Alternatively, the components of the fusion protein can be assembled using chemical methods of conjugation. Sources of the genes encoding the MHC molecules and the linkers can be obtained from DNA databases such as GenBank, as well as from published scientific literature in the public domain. In the case of MHC class I fusion proteins, the MHC fragment can be attached to the linker and .beta.2 microglobulin can be allowed to self-associate. Alternatively, the fusion protein gene can be constructed such that .beta.2 microglobulin is attached to the MHC fragment by a ether. In the case of MHC class II fusion protein, either the alpha or the beta chain can be attached to the linker and the other chain can be allowed to self-associate. Alternatively, the fusion protein gene can be constructed such that the alpha and beta chains are connected by a tether. Peptides can be prepared by encoding them into the fusion protein gene construct or, alternatively, with peptide synthesizers using standard methodologies available to one of ordinary skill in the art. The resultant complete fusion proteins can be administered by injection into the patient and can be repeated if necessary to provide a boosting reaction. Generally, the amount of fusion protein administered by injection for therapeutic purposes would range from about .1 .mu.g to about 100 mg per kilogram body weight. With a solid linker, the fusion protein could be injected if microparticles are used, or physically implanted if a larger linker is used.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 3. Document ID: US 6197302 B1

L1: Entry 3 of 3 File: USPT Mar 6, 2001

DOCUMENT-IDENTIFIER: US 6197302 B1
TITLE: Method of stimulating T cells

DEPR:
The fusion protein can be prepared by constructing a gene which encodes for the production of the fusion protein. Alternatively, the components of the fusion protein can be assembled using chemical methods of conjugation. Sources of the genes encoding the MHC molecules and the linkers can be obtained from DNA databases such as GenBank, as well as from published scientific literature in the public domain. In the case of MHC class I fusion proteins, the MHC fragment can be attached to the linker and .beta.2 microglobulin can be allowed to self-associate. Alternatively, the fusion protein gene can be constructed such that .beta.2 microglobulin is attached to the MHC fragment by a tether. In the case of MHC class II fusion protein, either the alpha or the beta chain can be attached to the linker and the other chain can be allowed to self-associate. Alternatively, the fusion protein gene can be constructed such that the alpha and beta chains are connected by a tether. Peptides can be prepared by encoding them into the fusion protein gene construct or, alternatively, with peptide synthesizers using standard methodologies available to one of ordinary skill in the art. The resultant complete fusion proteins can be administered by injection into the patient and can be repeated if necessary to provide a boosting reaction. Generally, the amount of fusion protein administered by injection for therapeutic purposes would range from about 1 .mu.g to about 100 mg per kilogram body weight. With a solid linker, the fusion protein could be injected if microparticles are used, or physically implanted if a larger linker is used.

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L5: Entry 4 of 4 File: USPT Feb 9, 1999

DOCUMENT-IDENTIFIER: US 5869270 A
TITLE: Single chain MHC complexes and uses thereof

DEPR:
The single chain MHC molecule may be either full length, i.e. the MHC molecule is associated with cellular domains and contains e.g. complete or substantial amounts (e.g. greater than 80% of the sequences) of transmembrane and/or cytoplasmic portions of an .alpha. or .beta. chain, or be truncated as discussed above for soluble expression. Such truncated and full length single chain MHC molecules may be produced as described above and in the examples for multiple polypeptide MHC complexes. For an MHC class II molecule, a full length molecule may have only one of the .alpha. and .beta. chains linked to transmembrane and cytoplasmic domains, preferably the .alpha. chain. A preferred full-length single chain fusion MHC class II complex comprises covalently linked in sequence: 1) the presenting peptide, 2) the class II .beta. chain lacking transmembrane and cytoplasmic domains, 3) a single chain linker sequence, and 4) the class II .alpha. chain containing transmembrane and cytoplasmic domains or a membrane anchor domain. A preferred soluble single chain fusion MHC class II complex comprises covalently linked in sequence: 1) the presenting peptide, 2) the class II .beta. chain lacking transmembrane and cytoplasmic domains, 3) a single chain linker sequence, and 4) the class II .alpha. chain lacking transmembrane and cytoplasmic domains.

DEPR:
Molecular weights of MHC fusion molecules as well as the empty and loaded MHC molecules of the present invention will vary, particularly depending on whether the molecule is soluble or full length (membrane bound). A soluble MHC class II fusion complex generally will have a molecular weight of greater than about 45 kDa, and mature .alpha. and .beta. chains without trans-membrane and cytoplasmic domains each will have a molecular weight of greater than about 20 kDa, more typically between about 21 to about 26 kDa. Typically, mature single-chain MHC class II molecules without trans-membrane and cytoplasmic domains will have a molecular weight of about 48 to about 50 kDa. For full length (membrane bound) molecules, mature .alpha. and .beta. chains generally will have a molecular weight of greater than about 25 kDa, preferably between about 26 and about 30 kDa. Typically, mature single-chain MHC class II fusion molecules with a single (linked to .alpha. or .beta. chain) transmembrane or membrane anchor domain will have a molecular weight of greater than about 49 kDa, preferably between about 50 and 52 kDa. All of the above mentioned molecular weights are by a SDS-PAGE determination.

DEPR:
The NSO murine B cell tumor line was transfected according to the Celltech Glutamine Synthestase Gene Amplification System Manual with minor modifications. This method uses electroporation to transfect mammalian cells with a vector (PEE-13) containing the coding region for the glutamine synthetase. Transfected cells have the ability to synthesize glutamine, thereby surviving without an exogenous supply. Selection of transformed clones was accomplished by isolating the cells that grow in glutamine-free medium. Briefly, 1.times.10.sup.7 NSO cells were washed twice in ice cold PBS and resuspended in 760 .mu.l of cold PBS. Forty .mu.g (40 .mu.l at 1 .mu.g/.mu.l)

of Sal I digested pJRS165.1 (See Example 12 above) plasmid DNA was added to the cells in an electroporation cuvette (0.4 cm). The cell/DNA mix was placed on ice for 5 minutes and the cells then electroporated using a Gene Pulser (Biorad) to deliver one pulse of 250 volts, 960 .mu.Fd. The pulsed cells were placed on ice for 2-5 minutes, removed from the cuvette, and added to 30 ml of non-selective medium (IMDM, 10% FBS, 2 mM L-glutamine, penicillin/streptomycin). Cells were plated in 96-well flat bottomed microtiter plates at 50 .mu.l/well (4 plates, cell suspension in 30 ml of medium as above; 5 plates, cell suspension diluted 1:4; 5 plates, cell suspension diluted 1:20) and then incubated with 5% CO.sub.2 at 37.degree. C. For the negative control, the same procedure of electroporation and plating was followed except that the DNA was omitted. The next day, 150 .mu.l of selective medium [IMDM, 10% dialyzed FBS, penicillin/streptomycin, nucleosides (6 .mu.g/ml A, G, C and U; 2 .mu.g/ml T), 60 .mu.g/ml glutamate and asparagine] was added to each well. The plates were fed with selective medium on a weekly basis by removing 100 .mu.l/well of used medium and adding 100 .mu.l/well of fresh medium, allowing the cells to gradually deplete the medium of all residual glutamine. Only those cells that have been transformed will survive, colonies becoming evident in 14-21 days. The colonies, or clones, were expanded and screened for expression of conformationally correct surface MHC Class II fusion complex, as detailed below.

DEPR:
Dendritic cells are professional, intradermal antigen presenting cells (APCs). The transformation of these cells (illustrated in this example) or other cells (such as exemplified in Example 13 above) with specific MHC class II fusion complexes can induce a peptide specific T-cell response. These APCs already bear the costimulatory molecules (i.e. B7-1) which provide the second activation signal to T-cells.

DEPR:
Two groups of BALB/c mice (9 mice per group) were injected i.d. on the shaved back with 100 .mu.l PBS containing 10 .mu.g of 1) pJRS165.1 carrying the encoding region of the murine OVA 323-339/I-A.sup.d MHC class II fusion gene under the control of the CMV promoter or 2) pABH1 carrying the encoding region of the murine HEL 74-86/I-A.sup.d MHCII fusion complex under the control of the CMV promoter as a control group. Four, 7 and 14 days after the injection the inguinal and paraaortic lymph nodes were collected. Lymph node cells were isolated and submitted to an OVA specific T-cell proliferation assay as follows. Cells were washed 3 times in complete medium (RPMI-1640, 10% FBS, 2 mM L-glutamine, penicillin, streptomycin, and 5.times.10.sup.-5 M 2-mercaptoethanol) and resuspended at 5.times.10.sup.6 cells/mL. One hundred microliters of the cell suspension were added to wells of a 96-well round bottomed microtiter plate. Dilutions of the OVA (323-339) peptide were prepared ranging from 0.08 .mu.g/mL to 10 .mu.g/ml and 100 .mu.L/well was added to the cells in triplicate. Background proliferation was determined by omitting the peptide. Plates were incubated with 5% CO.sub.2 at 37.degree. C., for 3-5 days. Wells were pulsed with 0.4 .mu.Ci of .sup.3 H-thymidine for 18 hours prior to termination of cultures and harvested using a Skatron Cell Harvester. Incorporation of .sup.3 H-thymidine into DNA as a measure of T-cell proliferation was determined using an LKB liquid scintillation spectrometer. The degree of peptide reactive T-cell proliferation was indicative of the T.sub.H -cell responses (i.e. of clonal expansion) that took place in the mice following immunization.